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EXAMINER	
STROUP,C	
ART UNIT	**PAPER NUMBER**
1633	4

DATE MAILED: 10/12/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary

Application No.	Applicant(s)
09/523,809	Murphy et al
Examiner	**Group Art Unit**
Stroup, Carrie	1633

☐ Responsive to communication(s) filed on _____

☐ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claim

☒ Claim(s) 1-30 is/are pending in the applicat

Of the above, claim(s) _____ is/are withdrawn from consideration

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) 1-30 is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☒ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 1

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

DETAILED ACTION

Claim Rejections - 35 USC § 112

1. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

2. Claims 1-30 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for tissue constructs produced by culturing dermal fibroblast cells derived from human neonatal foreskin on a collagen coated culture well in a defined culture medium which may further comprise a top layer of neonatal foreskin keratinocytes forming an epidermal layer, a tissue construct comprising transfected dermal fibroblasts expressing platelet derived growth factor, and a method of implanting said constructs on athymic mice, does not reasonably provide enablement for a construct comprising any transfected fibroblast or three separate cell layers or made in the absence of synthetic members and exogenous matrix components or a method of implanting said tissue constructs in all species. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. .

Applicant's claimed invention is to a cultured tissue construct comprising fibroblast cells cultured in the absence of exogenous matrix components or synthetic members to synthesize an extracellular matrix (ECM) comprising fibrillar collagen with a quarter-staggered 67nm banding pattern, decorin, glycosaminoglycans (GAG) (claims 1-7, 17, 18, 28, and 30); a cultured tissue construct comprising dermal fibroblast cells cultured in the absence

of exogenous matrix components or synthetic members to synthesize an extracellular matrix (ECM) comprising fibrillar type I and II collagen with a quarter-staggered 67nm banding pattern, decorin, glycosaminoglycans (GAG), fibronectin, and tenascin (claims 8, 17, 18, 28, and 30); a cultured tissue construct comprising two layers wherein the first layer comprises fibroblast cells cultured in the absence of exogenous matrix components or synthetic members to synthesize an extracellular matrix (ECM) comprising fibrillar collagen with a quarter-staggered 67nm banding pattern, decorin, glycosaminoglycans (GAG), and a second layer of epithelial cells (claims 9-15, 17, 18, 28, and 30); a method of making a cultured tissue construct comprising two layers wherein the first layer comprises fibroblast cells cultured in the absence of exogenous matrix components or synthetic members to synthesize an extracellular matrix (ECM) comprising type I and II collagen with a quarter-staggered 67nm banding pattern, decorin, glycosaminoglycans (GAG), tenascin, and fibronectin, and a second layer of keratinocytes on top of the first layer (claims 16, 17, 18, 28, and 30); and a method of making a cultured tissue construct comprising seeding fibroblasts cells on a porous membrane in a culture vessel to 80-100% confluence, stimulating said cells to produce ECM components of at least 30 microns thick in a second culture medium and comprising fibrillar collagen with a quarter-staggered 67nm banding pattern, glycosaminoglycans (GAG), and decorin (claims 19-23) or tenascin (claim 29), and seeding said matrix with epithelial cells on top of the first layer (claims 24-26) and wherein the seeding of fibroblasts is done at a specified density of cells/cm^2 topped with keratinocytes to form an epidermal layer (claim 27).

The specification fails to provide an enabling disclosure a tissue construct and the method of its production in the absence of synthetic members or extracellular matrix components during culturing conditions. The specification discloses the use of .05% poly-ethylene glycol (PEG) polymer in the culture media, which has been shown to promote in vitro processing of the soluble precursor procollagen produced by the culture cells (specification , pg 18, lines 17-20 & pg 31, line 15). The specification also discloses that the exemplified tissue constructs were produced in a Transwell six-well tray (pg 26, line 1), which according to the Corning Costar product catalog is coated with a collagen-treated

PTFE membrane that promotes cell attachment and spreading (see Transwell). In the absence of additional information on the exact composition of the culture plates, one of skill in the art would have required undue experimentation at the time of the invention to have made and used a tissue construct in the method disclosed by the specification without the use of PEG, or collagen-PTFE coated plates, and have it result in a construct with the same properties as demonstrated by the exemplified tissue constructs.

The specification is also not enabling for a tissue construct and the method of producing such, which exhibits an extracellular matrix comprising fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern and the presence of decorin. The specification discloses no assays, for example, immunohistochemical assays, for the identification of decorin, although this was disclosed for fibronectin, glycosaminoglycan, and tenascin, or that the collagen has a 67 nm banding pattern (pg 32, last para. - pg 33, para 1). And although the specification discloses that via utilization of microscopic methods it was shown that the construct displayed collagen with 67 nm banding pattern (pg 4, limes 10-11) and wherein a basement membrane was present at the junction of the dermal-epidermal junction, no evidence of such is disclosed (e.g., TEMs photographs) (pg 36, lines 9-17). In light of the unpredicatable nature of the tissue engineering art, which is plagued by results of bioengineered tissue which often does not display a morphology similar to human tissue (e.g. Minuth et al, abstract), submission of data demonstrating the above is appropriate.

The specification also fails to provide an enabling disclosure for a cultured tissue construct or the method of producing such utilizing cells cultured in chemically defined media and with no non-human components. The specification discloses that a chemically defined media is free of undefined animal organ or tissue extracts, for example, serum or proteins, especially those of non-human origin as disclosed in US Patent 5,712,163 Parenteau et al, which is incorporated by reference (specification, pg 12, lines 17-21, and pg 14, lines 2-3). The specification discloses, though, examples in which 2% newborn calf serum is utilized (e.g., pg 31, line 9) which resulted in formation

of tissue comprised of collagen, glycosaminoglycan, fibronectin, and tenascin (pg 32, last para-pg 33, para 1). It would have required undue experimentation by one of skill in the art at the time of the invention to have made and used a tissue construct in the method disclosed by the specification without the use a culture medium comprising non-human components and have it result in a construct with the same composition and properties as the exemplified tissue constructs.

The specification fails to provide an enabling disclosure for a cultured tissue construct comprising cultured cells from the dermal papilla of hair follicles which are grown to produce a layer of extracellular matrix comprising collagen exhibiting 67 nm banding pattern, decorin, and glycosaminoglycan (GAG) (claims 4, 5, 13, and 14). Example 12 of the specification discloses that an extracellular matrix is first formed with foreskin fibroblasts and then locally seeded with dermal papilla cells and in turn seeded with keratinocytes. The specification also discloses that the resulting skin exhibited a dermal layer consisting of fibroblasts surrounded by endogenously produced matrix and localized areas of dermal papilla cells and a continuous stratified layer of keratinocytes, but does not disclose that collagen, decorin, or GAG existed within the tissue (pg 52, lines 1-5).

The specification also fails to provide an enabling disclosure for a tissue construct comprised of three cell layers (claim 15). The specification does not provide an exemplifying disclosure for a method of making a three layer tissue construct (it is noted that the above referenced Example 12 does not technical comprise three layers of cells, but two layers with seeding of a third cell type within the first layer). The specification fails to disclose essential details such as the culture media and conditions, the timing of the application of the third layer, e.g. after stratification of the second layer, or the amount of cells per type of tissue design. Instead the specifications's disclosure is limited to generalities, such as "On the second cell-matrix construct, a third seeding of a third cell type is seeded and cultured under sufficient conditions to produce the third layer" (pg 26, lines 17-19). Said "sufficient conditions" are not defined. In light of the unpredictability in the art of tissue engineering, it would have required undue experimentation by one of skill in the art

to have made a three layered tissue construct which would have displayed the composition of the claimed tissue construct, e.g. collagen, decorin, and GAG, and been displayed cell morphology and mechanical properties similar to that seen in human tissue.

The specification fails to provide an enabling disclosure for any genetically modified fibroblast cells other than the exemplified human dermal fibroblasts transfected to express platelet derived growth factor (Example 6, pg 33-34). The specification incorporates by reference to Sambrook et al, Molecular Cloning (1989) the essential teachings of genetically engineered cells. The specification does not disclose essential teachings such as the type of vector with promoter or the method of transduction to ensure high level transgene expression. Gene therapy is a highly unpredictable art as demonstrated by Schinstine et al who showed that long-term transgene expression did not occur when fibroblasts genetically modified with a retroviral vector were transplanted into human brain tissue (Schinstine et al, 1997). Likewise, Garlick et al teach that not all transduction methods are equivalent, for example, the use of micro-injection in gene delivery is limited to "a relatively small number of cells due to the exacting technical demands of micro-injection"(Garlick et al, pg 206, col 1, para 2). Garlick et al also teach that the successful use of retroviral vectors for *ex vivo* transduction of cells, such as keratinocytes, depends largely on the viral titers utilized, wherein titers greater than $5*10^6$ CFU/ml resulted in transduction of all clonogenic cells, while titers in the range of 10^5 transduced approximately 5% of the clonogenic cells (pg 206, col 2, para 3). The specification of the pending application fails to provide the any specific teachings for the method of making genetically modified fibroblasts other than that exemplified in Example 6, the result of which would be undue experimentation by the artisan to transduce fibroblasts ex vivo, transplant them into a wound site, and have a sufficiently high level of transgene expression which would result in any useful therapy.

The specification does not reasonably provide enabling disclosure for a tissue construct comprising fibroblasts derived from tendon, lung, urethra, umbilical cord, corneal stroma, oral mucosa, and intestine, or a bilayer construct

comprising corneal epithelial cells, or epithelial cells from oral mucosa, esophageal epithelial cells, and uroepithelial cells, or a method of transplantation or implantation in a patient. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. The art of tissue engineering is highly unpredictable, as previously stated, for which the currently pending application does not provide teachings to overcome. For example, Parenteau et al teach that different cell types utilized in tissue constructs require different quantity of cells during the cell culturing procedure (e.g. col 21, lines 27-31 and Table 1). The specification also provides exemplification for the use of human tendon fibroblasts, corneal keratinocytes, and buccal fibroblasts, Examples 5, 9, 17, respectively, yet the specification does not disclose that the resulting construct comprised collagen of 67 nm banding pattern, decorin, or GAG as claimed. Additionally, the specification discloses the grafting of a fibroblast construct onto athymic mice (Example 7), said results do not directly correlate with human patients, especially in light of the high incidence of graft rejection by the host immune system, nor was said resulting tissue shown to comprise collagen, decorin, GAG or any other specific extracellular matrix component. In light of the unpredictability in the art and the absence of teachings within the specification, such as the specific quantity of fibroblast and epithelial cells to use per type, and methods of overcoming immune response in human patients, it would require undue experimentation by one of skill in the art to have utilized the disclosed specification to make and use tissue constructs of cells types other than fibroblasts from neonate foreskin and derma and keratinocytes, and to transplant said constructs into any animal other than athymic mice.

3. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 1-30 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-30 are unclear as to the metes and bounds of "exogenous matrix components" and "synthetic members". Does "exogenous matrix components" refer to anything other than extracellular matrix components such as collagen? Does synthetic members refer to any non-naturally occurring polymer including polyethylene glycol (PEG)?

Claims 7 and 21 are unclear as to the metes and bounds of a culture medium containing "no non-human components". Does this include any chemical or protein, to include essential and non-essential amino acids, which are not produced in the human body or required for its proper functioning?

Claims 19-23 and 24-26 are unclear as to the steps involved in "stimulating" the fibroblast cells to synthesize, secrete, and organize extracellular matrix components. Does this comprise the addition of a specific nutrient or mechanical manipulation of the cells in culture?

Claims 1-18, 28 and 30 are unclear as to the metes and bounds of cultured "under conditions to produce a layer of extracellular matrix". Do these "conditions" comprise a specific culture medium, duration, and temperature of culturing?

Claim Rejections - 35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. Claims 1-3 , 6-12, and 19-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bell, E. (US Patent 4,485,096), Parenteau et al (US Patent 5,712,163), Sand, BJ (US Patent 5,618,284) and Holbrook et al (1993) and Biegel et al (1994).

Applicants' claimed invention is to a cultured tissue construct comprising fibroblast cells, such as dermal fibroblasts derived from neonate male foreskin, grown under conditions on a porous membrane to produce a layer of extracellular matrix comprising type I and II fibrillar collagen, glycosaminoglycan, decorin, fibronectin, and tenascin and wherein said cells are cultured in the absence of exogenous matrix components in a chemically defined media, and which may further comprise a second layer of epithelial cells, such as keratinocytes (claims 9-12, 16) ; and a method of transplanting or implanting said tissue construct into a patient (e.g. animal) (claim 28). The claimed invention also includes a method of producing a cultured tissue construct comprising seeding fibroblasts cells at a density between $1*10^5$-$6.6*10^5$ cells/cm^2 on a porous membrane in a first cell membrane to 80-100% confluence, stimulating the cells to synthesize an extracellular matrix in a second culture medium until a matrix of 30-100 microns thick and comprising collagen, decorin, and GAG is formed, and with or without the seeding of a second layer of epithelial cells, such as keratinocytes (claims 19-27).

Bell, E teaches the use *in vitro* of human foreskin and dermal fibroblasts cultured in Falcon bacteriological dishes comprising McCoy's 5a medium, Fetal Calf Serum, NaOH, and a collagen solution to form a contractable, transplant tissue and wherein a layer of keratinocytes may be added *in vitro* (claims 15 & 16; and Example 1, col 8, lines 39-55, col 3, lines 28-30). Bell also teaches the method of tissue transplantation in guinea pigs and rats (e.g. Examples 10 and 11). Bell et al does not teach the use of chemically defined media, the molecular composition of the differentiated tissue, e.g. collagen, decorin, and GAG, or the use of said procedure in the absense of exogenous matrix components (e.g. collagen).

Parenteau et al teach the use of a chemically defined cell culture medium, which by definition are absent of undefined proteins from protein supplements such as serum, and wherein the cell culture systems comprise said cell culture medium and a substrate for the cells, such as glass or plastic, and in the absence of exogenous matrix components or synthetic membranes which resulted in the prolonged growth and differentiation of cells, such as keratinocytes,(e.g. col 1, lines 35-55, claims 20 and 24). Parenteau et al also teach the method of producing skin equivalents grafts in vitro utilizing keratinocytes and dermal (fibroblast) equivalents (example 6, col 25, line 61-col 26, line 14), and the use of a sequential two culture medium process in the absence of a substrate which showed only a slight decrease in plating efficiency in comparison to those that were grown on a collagen substrate (e.g. Table 5, col 5).

Sand, BJ teaches that human type-1 collagen molecule consists of chains of 300 nm triple helixes joined by 67 nm uncoiled bonds (col 10, lines 32-33).

Holbrook et al teach that the dermal matrix of connective tissue is comprised of collagen, of which 80-90% is type I and 8-12% is type III, glycosaminoglycan, fibronectin, and tenascin (pg 117, col 1, para 3 & pg 119, col 1, para 1 and 3).

Biegel et al teach the use of the Transwell filters coated with hydrated collagen gels for the use in growing endothelial cells in vitro which resulted in monolayers growing until confluency and exhibiting biochemical, morphological, and electrophysiological properties reflective of cells in vivo (abstract).

In light of Bell, Parenteau, Sand, Holbrook and Biegel et al it would have been obvious to one of ordinary skill in the art to make a cultured tissue construct comprising fibroblast cells, such as neonate male foreskin or dermal, grown under a sequential cell culture conditions on a Transwell plate coated with collagen to produce a layer of extracellular matrix comprising type I and III fibrillar collagen, glycosaminoglycan, decorin, fibronectin, and tenascin and wherein said cells are cultured in the absence of exogenous matrix components in a chemically defined media

containing no non-human components and comprising a second layer of epithelial cells, such as keratinocytes; and utilizing said construct for transplanting in an animal model. Note the percentage of cell confluence, the thickness of the resulting matrix, and the density of the seeded cells are rate effective variables which one of ordinary skill in the art could readily ascertain through routine experimentation. One would have been motivated to utilize a tissue construct in the absence of exogenous matrix components to provide an efficacious method of dermal regeneration which did not require the construction of a biodegradable matrix , and to utilize a chemical defined medium to optimize tissue differentiation and growth (Parenteau et al, col 1, lines 35-55). One would also have been motivated to use a porous membrane, such as Transwell plates, because the layer of collagen (or polycarbonate membrane) on the plates would allow for the efficacious adhesion and differentiation of fibroblast cells, especially in light of the absence of an exogenous extracellular matrix scaffold. There would have been a reasonable expectation of success because Bell demonstrated that tissue constructs could be generated utilizing a base layer of fibroblasts with a top layer of keratinocytes to generate full thickness skin grafts and done in the absence of three dimensional matrices (e.g. Bell, claim 16) and because Parenteau et al had demonstrated the successful use of tissue constructs comprising keratinocytes using said defined culture medium and in a sequential two step culture system and because Biegel et al had demonstrated the sucessful use of the Transwell system for in vitro growth and differentiation of endothelial cells into tissue (e.g. Parenteau et al, Example 5, col 24, lines 35-40, & Biegel et al, abstract).

7. Claims 1, 4, 5, 9, 13, and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Jahoda et al (1993) in view of Parenteau et al (US Patent 5,712,163).

Applicants' claimed invention is to a cultured tissue construct comprising dermal papilla of hair follicles which are localized on and grown to produce an extracellular matrix and comprising a second layer of epithelial cells (claim 9) and wherein the cells are cultured in the absence of exogenous matrix components or synthetic members.

Jahoda et al teach that the transplantation of dermal papilla cells in rat ear wounds resulted in the production of hair growth in comparison to a control of transplanted skin fibroblasts, which resulted in no new hair growth (abstract and pg 585, col 1, para 1-3 and Table 1). Jahoda et al does not teach the use of a cultured tissue construct system grown in vitro to produce extracellular matrix components.

Parenteau et al teach the use of a chemically defined cell culture medium, which by definition are absent of undefined proteins from protein supplements such as serum, and wherein the cell culture systems comprise said cell culture medium and a substrate for the cells, such as glass or plastic, and in the absence of exogenous matrix components or synthetic membranes which resulted in the prolonged growth and differentiation of cells, such as keratinocytes,(e.g. col 1, lines 35-55, claims 20 and 24). Parenteau et al also teach the method of producing skin equivalent grafts in vitro utilizing keratinocytes and dermal (fibroblast) equivalents (example 6, col 25, line 61-col 26, line 14), and the use of a sequential two culture medium process in the absence of a substrate which showed only a slight decrease in plating efficiency in comparison to those that were grown on a collagen substrate (e.g. Table 5, col 5).

In light of Jahoda and Parenteau et al, it would have been obvious to one of ordinary skill in the art at the time of the invention to create a cultured tissue construct comprising dermal papilla cells with fibroblast cells and with or without a top layer of epithelial cells, such as keratinocytes. One would have been motivated to do this to provide a method of producing a tissue construct that could be used to generate new hair growth (Jahoda et al, Table 1). There would ~~be~~ have been a reasonable expectation of success because Jahoda et al demonstrated the ability to culture dermal papilla cells in MEM containing fetal bovine serum and L-glutamine and then transplant them into rats for successful production of hair and the culturing and implantation of fibroblasts for successful production of dermal skin, while Parenteau et al had demonstrated the ability to culture keratinocytes in the absence of exogenous matrix components and synthetic membranes to produce differentiated, stratified tissue (Parenteau et al, abstract).

No claim is allowed. It is noted, though, that the prior art of record is free of fibroblasts genetically engineered to express platelet derived growth factor for use in a tissue construct.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carrie Stroup whose telephone number is (703) 306-5439. The examiner can normally be reached on Monday through Friday from 8:30 AM to 6:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, John LeGuyader, can be reached at (703) 308-0447. The fax number for this Group is (703) 308-0294.

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